EFFECT OF COMPLETELY EXPRESSED HINDMILK-RICH FEEDS IN INCREASING WEIGHT GAIN OF PRE-TERM NEWBORNS

Dissertation Submitted for

MD DEGREE EXAMINATION
BRANCH VII - PAEDIATRIC MEDICINE

INSTITUTE OF CHILD HEALTH
AND
HOSPITAL FOR CHILDREN
MADRAS MEDICAL COLLEGE
THE TAMILNADU Dr.M.G.R. MEDICAL UNIVERSITY
CHENNAI.

SEPTEMBER 2006

CERTIFICATE

Certified that this dissertation entitled **" EFFECT OF COMPLETELY EXPRESSED HINDMILK-RICH FEEDS IN INCREASING WEIGHT GAIN OF PRE-TERM NEWBORNS "** is a bonafide work done by **Dr. K. JEGEN M.D.,** Post Graduate Student of Pediatric Medicine, Institute of Child Health and Hospital for Children, Egmore, Chennai - 600 008, during the academic year 2003 - 2006.

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DECLARATION

I declare that this dissertation entitled **"EFFECT OF COMPLETELY EXPRESSED HINDMILK-RICH FEEDS IN INCREASING WEIGHT GAIN OF PRE-TERM NEWBORNS"** has been conducted by me at the Institute of child health and Hospital for Children, under the guidance and supervision of **Prof. Dr. Meer Mustafa Hussain, MD., DCH., Ph.D.,** Head of the Department of Medical Neonatology, and unit chief M – I **Prof. Dr. Bhagavathy, MD., DCH.,.** It is submitted in part of fulfillment of the award of the degree of M.D (Pediatrics) for the September 2006 examination to be held under The Tamil Nadu Dr. M.G.R Medical University, Chennai. This has not been submitted previously by me for the award of any degree or diploma from any other university.

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SPECIAL ACKNOWLEDGEMENT

My sincere thanks to **Prof. Dr. Kalavathi Ponniraivan,** **B.Sc.**, **M.D.**, the Dean, Madras Medical College for allowing me to do this dissertation and utilize the institutional facilities.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to **Prof. Dr. Mangayarkarasi Senguttuvan M.D., D.C.H.,** Professor of Pediatrics, Director and Superintendent of Institute of Child Health and Hospital for Children for permitting me to undertake this study.

I am extremely thankful to **Prof. Dr. Meer Mustafa Hussain M.D., D.C.H., Ph.D.,** Professor of Pediatrics and Head of the Medical Newborn Unit, for his guidance, invaluable help, encouragement and support throughout the study.

I am extremely thankful to **Prof. Dr. Bhagavathy, M.D., D.C.H.,** Professor of Pediatrics and our unit chief for her guidance, invaluable help, encouragement and support throughout the study.

I am extremely thankful to **Asst. Prof. Dr. J. Kumutha, M.D., D.C.H.,** Assistant professor of pediatrics for her guidance, invaluable help, encouragement and support throughout the study.

I would like to thank our unit Assistant Professors, **Dr .K. Umayal, M.D., D.C.H., Dr. S. Parivarthini, M.D., D.C.H., Dr. K. Devi Meenakshi, M.D., DCH., and Dr. B. Satyamoorthy, M.D., DCH.,** for their valuable guidance and support in doing this study.

I extend my sincere thanks to **Dr. Kothainayagai, M.D., D.C.H.,** and **Dr. P. Ramachandran M.D., D.C.H.,** Registrars for their valuable suggestion and guidance in doing this work.

I would like to thank **Mrs. Basilea Watson**, statistician, Madras Medical College, for her invaluable help.

I sincerely thank all the children and their parents who have submitted themselves for this study without whom this study would not have been possible.

CONTENTS

INTRODUCTION

Following birth newborns rapidly adapt from a relatively constant intrauterine environment and supply of nutrients to intermittent feedings of milk after birth. Preterm infants are however at an increased risk of potential nutritional compromise. These infants are born with limited nutrient reserves, immature pathways for absorption and metabolism, and increased nutrient demands[1]. In addition, medical and surgical conditions commonly associated with prematurity frequently alter nutrient demands and complicate adequate nutrient delivery. As mortality rates for these high risk newborns continues to improve, optimizing nutritional care beginning in the immediate postnatal period, has become an important topic of clinical research. Current research has suggested that earlier, more aggressive nutritional intervention is desirable.[1] Nutritional practices vary dramatically among Neonatal Intensive Care Units (NICUs). In many institutions, nutrition is introduced only gradually over the first weeks of life because of concerns of nutrient intolerance by the very preterm or ill infant who is fed intravenously and the risk of necrotizing enterocolitis (NEC) in preterm infants who are fed enterally. Often this period of nutritional deficiency is accepted as inevitable in this population. However, such a strategy of cautious nutrition might lead to a period of early malnutrition from which the neonate has a prolonged recovery and may have long-term adverse consequences. In many neonates this period of early nutritional deprivation need not be inescapable[2].

BREASTFEEDING THE NEWBORN

The newborn has only three essential needs: the human warmth of the mother's arms, the certainty of the mother's presence, and the milk of her breasts. Breastfeeding satisfies all three**.** Maternal breastfeeding is the most natural and safe way to feed a small child. Breastfeeding provides a unique combination of proteins, lipids, carbohydrates, minerals, vitamins, enzymes and living cells, as well as known and unquestionable nutritional, immunological, psychological and economic benefits. The nutritional composition of breast milk, which possesses the exact proportion of nutrients for good development of the human brain, differs from the milk of other mammals, which contains large amounts of protein for the rapid growth of the species[3]. Human milk is a complex species-specific biological fluid, adapted throughout human existence to perfectly satisfy the nutritional and immunological needs of the child. Mature human milk is a homogeneous mixture consisting of three fractions: emulsion (fat droplets), suspension (casein micelles) and solution (water soluble components)[3]. Different energy contents have been described to mature milk in the literature, ranging from 65.7 kcal/dl, 71 kcal/dl to 75 kcal/dl[3]. Dynamic variations in the nutritional components of human milk exist, which depend on the stage of lactation, the time of day, period of feeding, maternal nutrition and age, and gestational age of the infant, as well as individual aspects of each lactating mother[4]. Fat is the most variable component of maternal milk, corresponding to 3-4 g/dl, and represents the main energy source for the newborn, providing 35 to 50% of its daily needs. Almost all lipids in human milk are present in the form of droplets, which permit stabilization of the emulsion and increases the bioavailability of liposoluble components. Lipid components include triglycerides, phospholipids and cholesterol, as well as free fatty acids, and are derived from circulating fat originating from the diet and from maternal body reserves, or are synthesized from glucose in the breast itself[5]. Human milk shows a high concentration of cholesterol, with its plasma concentration being higher in breastfeeding infants than in those fed with artificial milk. There is evidence showing the beneficial effect of a higher cholesterol concentration during this phase of life based on its better bioavailability to the developing brain and for maintaining low and appropriate blood levels of cholesterol during adult life[28]. Digestion of triglycerides in the neonate is facilitated by a combination of enzymes secreted by the gastrointestinal tract of the infant, (lingual lipase, gastric lipase, pancreatic lipase). Bile salt-stimulated lipase provided by the human milk fat droplets themselves are activated by bile salts in the duodenum and help in the digestion of fat[29]. Human milk represents the best source of essential fatty acids. A great difference between human and artificial milk concerns the concentration of long-chain polyunsaturated fatty acids, which are essential for the normal development, with emphasis on docosahexaenoic acid which is important for the development of the brain and retina, and arachidonic acid as a precursor of prostaglandins and leukotrienes. The reserves of these fat elements are limited at birth, especially in premature newborns, and rapidly decrease when lacking in the diet[5].

USE OF HUMAN MILK IN PREMATURE INFANTS

The recommendation of breastfeeding for the premature newborn has been defended on the basis of the immunological properties of human milk, its role in gastrointestinal maturation, formation of the mother-child bond, and improvement in neurobehavioral performance, shown by breastfed children. Feeding of premature infants with human milk has been suggested to have short term, medium term and long term benefits. Premature infants show a better suction-deglutition coordination during breastfeeding. The transcutaneous partial oxygen pressure levels, oxygen saturation and body temperature have been shown to be higher than those observed during bottle feeding[30], confirming that breastfeeding is a more physiological process. Human milk exerts a protective effect on premature infants against necrotizing enterocolitis. Lucas and Cole[7] observed a six to ten times higher incidence of this disorder in premature newborns who exclusively received artificial milk compared to those fed human milk. Likewise, the incidence of any infection, including sepsis and meningitis, has been found to be significantly lower among very low birth weight newborns fed human milk than among those receiving exclusively artificial milk[11,12]. During the hospital stay of the mother and the premature infant, the mother produces antibodies (Enteromammary immunity) against nosocomial microorganisms that occur at the neonatal unit, a fact important for the prevention of infection in the neonate during the hospital stay[27]. These data were confirmed by the observation of a significant reduction in the incidence of severe infections in patients submitted to the Kangaroo Mother Care, in which the newborn is maintained in a vertical position, in prone decubitus, against the mother, leading to early and growing contact between them, compared to patients treated by the traditional method[31]. Maternal milk has been found to protect premature infants with a family history of atopy against allergy, especially in terms of the incidence of eczema. After 18 months of age, children who received artificial milk showed a higher risk of developing atopy than those receiving human milk from a milk bank[32]. Omega 3 fatty acids are essential for a normal development of the retina, especially in very low birth weight newborns. These fatty acids, together with antioxidant substances such as vitamin E, ß-carotene and taurine, might explain the protective effect of human milk on the development of retinopathy of prematurity, since the incidence and severity of this disease are significantly lower in premature infants exclusively fed with maternal milk or who ingested at least 80% of their milk in the form of human milk[33]. Lucas et al[7] observed advantages in terms of cognitive performance, assessed at 18 months and between 7.5 and 8 years of age, in preterm children fed human milk. This study was carried out with scientific rigor, with adjustment for factors such as social class and educational level of the mother, days of mechanical ventilation, and gender. Since most premature newborns received milk by gavage, there is evidence that the best psychomotor development was due to the milk itself and not to the quality of mother care, supporting the statement that human milk is the optimal diet for the developing brain[14]. Epidemiological evidence suggests that feeding human milk is related to a lower incidence of re hospitalization of premature infants, even after the introduction of food supplements. The biological benefits of maternal milk render it an excellent food for premature newborns, even when considering that eventual losses of nutrients due to collection, processing and storage and to the method used to offer human milk to patients of neonatal units[13] might be responsible for the lower growth rate observed for these newborns compared to those receiving artificial milk[34]. Although neonatal growth performance is better in preterm infants fed premature formulas, this is not true for measurements of weight, height, head circumference and cutaneous fold around nine months and eight years of age, which were found to be similar irrespective of whether the diet received was preferentially maternal milk or exclusively artificial milk[35]. The diet of choice for premature newborns is the milk of their own mothers. In the case the child is unable to directly suck at the breast, he should receive manually expressed milk. The feeding strategy, which results in a better weight gain of premature newborns, is to offer hindmilk that contains up to three times more fat than foremilk. The milk produced by mothers of premature newborns during the first four weeks post-partum contains a higher concentration of nitrogen, proteins with immunological functions, total lipids, medium chain fatty acids, vitamins A, D and E, calcium, sodium, and energy than the milk of mothers of term infants. Therefore, milk obtained from the milk bank pool might be nutritionally inadequate for premature infants[36]. Neonatologists need not only to be convinced of the multiple advantages of breastfeeding and of the possibility to feed human milk to premature newborns, but should also integrate the management of lactation into the planning of therapeutic actions for these patients. In view of the enormous advances in neonatology since the end of the sixties, a population of premature very low birth weight newborns has arisen, which, until recently, did not survive and which, due to their immaturity and clinical complications, is subject to childhood malnutrition and rehospitalization[1]. Studies making breastfeeding viable for this risk population have unquestionable social importance since these children, due to their immunological impairment, would particularly benefit from breastfeeding, especially in developing countries with high infant mortality rates and endemic malnutrition. Although desirable, a low success rate of breastfeeding has been observed among mothers of premature neonates[37], since many hospital barriers to breastfeeding still exist, especially in high risk neonatology services, and weaning from the breast frequently occurs even before discharge of the premature newborn from the high risk nursery. During the stay at the neonatal unit, many mothers perceive that nursing their child is the only thing that they can effectively do to collaborate in the recovery of the premature newborn; however, only few mothers are able to initiate and maintain an adequate milk production without qualified help and family support. Breastfeeding premature infants represents a challenge. The premature newborn shows physiological and neurological immaturity, muscle hypotony and hyperreactivity to environmental stimuli, and remains alert for short periods of time only. However, despite its inadequate suction-deglutition-respiration control, a premature newborn is able to feed at the breast as long as appropriate help is provided[38]. The success of the work of promotion, protection and support for breastfeeding in premature infants depends on an interdisciplinary team consisting of neonatologists, lactation consultants, nutritionists, occupational therapists, physiotherapists, and social assistants, who should be prepared to integrate the clinical hospital management of lactation within the routine functioning of high risk nurseries. Even better would be to permit that hospitalized premature newborns be neither deprived of the presence of their mothers nor of breastfeeding.

NUTRITIONAL GOALS OF THE PRETERM INFANT

Just as pediatric patients are not "little adults," the preterm infant clearly has increased nutritional needs compared to the infant born at term. Furthermore, extremely low birth weight (ELBW) infants (those who weigh <1000g at birth) have even greater metabolic demands. Traditionally, nutritional support of ELBW infants has targeted establishing fetal growth by addressing the changing nutritional requirements of these infants[6,7] Providing appropriate nutrition for growth and development is a cornerstone of the care of preterm infants. Early postnatal nutrition during this critical period of brain growth may have a substantial impact on clinically important outcomes, including long term neurodevelopment[6,7]. Preterm infants, especially those who have been growth restricted in utero, have fewer nutrient reserves at birth than term infants. Additionally, preterm infants are subject to physiological and metabolic stresses that can affect their nutritional needs, such as respiratory distress or infection. An international consensus group has recommended nutritional requirements for preterm infants (Tsang 1993)[39] These recommendations are based on data from intrauterine growth and nutrient balance studies and assume that the optimal rate of postnatal growth for preterm infants should be similar to that of normal fetuses of the same postconception age. In practice, however, these target levels of nutrient input are not always achieved and this may result in important nutritional deficits[39].

Despite the many medical advances in the past twenty years which have improved the survival of preterms infants, their postnatal growth continues to lag. In fact, recent studies of postnatal growth in large numbers of infants cared for in modern NICU's have shown a common difficulty in achieving intrauterine growth rates, but significant internursery variability[40]. Growth was most accelerated in those units that instituted enteral feedings early and advanced total nutrition aggressively. Advances in the care of low birth weight infants have led to the survival of increasing numbers of very premature infants. Nutritional management plays an important role in the immediate survival and subsequent growth and development of these infants. Undoubtedly, mothers milk is the gold standard for the feeding of full-term infants. Breastfeeding is recommended throughout the first year. However, for premature infants, in particular those born with a very low birth weight (<1500 g), the acceptance of mother's milk as the feeding of choice is not universal[39]. Dilemmas exist over special formulas that have been designed to meet the unique needs of these infants[40]. Defining the nutritional goals is crucial when choosing the appropriate feeding for preterm infants. The current standard for nutritional management of preterm infants is to achieve a postnatal growth that approximates the in-utero growth of a normal fetus at the same post conceptional age[39]. This includes not only anthropometric measures, but also changes in body composition and retention of individual nutrients. However, it is debatable what constitutes optimal growth, and there is no compelling reason to suggest that extrauterine and in-utero growth should be the same. Commonly used charts showing intrauterine growth are static, and contain cross-sectional data obtained from infants born at different gestational ages[36]. In addition, the data are derived from pregnancies that cannot be considered normal since maternal conditions resulting in fetal loss or preterm delivery often affect fetal growth[36]. The fetus is intravenously fed and has little net energy expenditure. The main sources of nutrition in-utero are glucose and amino acids. Postnatally, the diet has a comparatively high fat content; fat accumulation in the preterm infant is far greater than in the equivalent gestation fetus. Energy expenditure for thermoregulation may be high[38]. The infant's hormonal response to intravenous and enteral feeding is also different. The premature infant is unique. From medical and practical standpoints, the weight change in a very low birth weight (VLBW) infant during the first 1 to 2 weeks of life reflects more fluid management than tissue deposition[11]. Fluid administration generally is targeted to cover insensible water losses. Relative fluid restriction usually is practiced to prevent development of patent ductus arteriosus, with some centers allowing weight to decrease by as much as 15 to 20% below birth weight in the first days of life[10]. At the same time, because of concerns over intolerance of both intravenous and intragastric feeding during the stresses arising from birth and intercurrent illness, parenteral and enteral nutrition are commonly introduced gradually over a period of days. Enteral feeding is generally withheld in the first week of life[11]. Thus, rates of weight gain that would occur in-utero would not be expected to occur for at least the first 1 to 2 weeks of life[12] Additionally, diuretic administration, steroid use and other complications may adversely affect postnatal weight gain. While achieving in-utero body composition at comparable gestational age may, in theory, be a reasonable nutritional goal, attaining specific weight gains is not indicative of growth. A pragmatic approach to achieving optimal growth is to allow the infant to fulfill his genetic potential, and grow at the fastest attainable rate, using intrauterine data as broad guidelines, without imposing stress on the infant's immature metabolic and excretory system[11]. The search for a true gold standard for assessing nutritional outcome of preterm infants has seen an expansion beyond the traditional intrauterine-based short term growth and nutrient retention rates toward a broader, and possibly life-long, range of outcomes[15]. An alternative goal of early nutritional management is the prevention of neonatal morbidity including feeding intolerance, necrotizing enterocolitis, and sepsis. Furthermore, the early diet should have a favorable impact on longterm outcomes such as optimizing neurodevelopmental outcome; reducing the rates of allergy and atopic diseases[12]; and preventing or reducing adult-onset diseases eg. diabetes mellitus, hypertension, heart disease and hypercholesterolaemia[13]. As our nutritional end points shift, the suitability of the preterm infants' own mothers milk may become more apparent.

NUTRITIONAL SUITABILITY OF HUMAN MILK FOR THE PRETERM

Human milk has all the essential nutrients to meet the nutritional demand; has a low renal solute load, which is important for the infant's kidneys; and is easy to digest and absorb. In addition, the composition of human milk changes to meet the needs of the growing and developing infant. The nutrients from human milk are also more bioavailable than nutrients from other sources. The benefits of the predominant whey protein fraction of human milk have been investigated. Premature infants fed with whey-dominant milk manifest less imbalance in their plasma amino acid concentrations of phenylalanine, tyrosine and methionine than those given casein-dominant milk[13]. However, the type of proteins contained in the whey fraction differs between human and cow's milk. The major human whey protein is alpha-lactalbumin. In cow's milk, this is beta-lactoglobulin–also the protein thought to be responsible for cow milk protein allergy and colic[41]. Lactoferrin, lysozyme and secretory immunoglobulin A are specific human whey proteins involved in host defence mechanisms, but these are present only in trace quantities in cow milk[13]. Human milk fat digestion and absorption are facilitated by the complex organisation of the human milk fat globule: the pattern of fatty acids that are high in palmitic, oleic, linoleic and linolenic acids; their distribution on the triglyceride molecule; and the presence of bile salt-stimulated lipase. The lipid system in human milk is particularly structured to suit the preterm infant. The mixture of fatty acids in infant formulas differs from that in human milk[11]. Generally, commercial formulas have either modified their fat blends in an attempt to mimic the fat absorption in human milk, or have a greater quantity of medium chain fatty acids to compensate for the absence of a total lipid system characteristic of human milk. Besides promoting fat absorption, the pattern of essential fatty acids in human milk may benefit the developing premature infant. The very long chain fatty acids, arachidonic acid and docosahexaenoic acid, are found in human but not cow's milk[11]. They are components of phospho-lipids found in brain, retina and red cell membranes. Functionally, they are associated with cognition, growth, and visual function. The carbohydrate composition of human milk is important as a nutritional source of lactose and oligosaccharides. Early milk feeding stimulates endogenous lactase activity and premature infants can absorb over 90% of the lactose in human milk[15]. The presence of a small proportion of unabsorbed lactose is thought to be a normal physiological effect of human milk, giving rise to a softer stool consistency, more nonpathogenic bacterial faecal flora, and improved mineral absorption. The oligosaccharides are carbohydrate polymers whose structure mimics specific bacterial antigen receptors. By preventing bacterial attachment to the gut mucosa, oligosaccharides are important in host defence of the infant[11]. As research continues, we have become acutely aware that the milk from preterm mothers is significantly different from that of mothers who deliver at term. The composition of human milk also changes in relation to how prematurely the infant was born. According to Lawrence and Lawrence, "Neonatologists have not reached a consensus on the real goals of feeding for the premature[37]." Lucas feels that it is unnatural to keep any baby from oral feedings, regardless of gestational age[39]. The fetus starts swallowing amniotic fluid very early in gestation. At term the baby may be swallowing up to 500 ml per day [of amniotic fluid] which actually provides as much as 3 grams of rotein per day, and may help the gut mature (Lawrence and Lawrence)[37].

Human milk feeding, even in small quantities, for the very low birth weight (VLBW) infants, provides benefits that we are only beginning to understand. Preterm mothers' milk contains amino acids and fat blends that aid physical growth. Breastmilk components have overlapping or interdependent functions. The fat globules in preterm milk are smaller thus aiding in their absorption directly from the immature gut. Premature infants lack the enzymes and bile salts needed for digestion of fats. However, they absorb 95% of the fats in human milk compared with 83-85% of artificial milks[10]. The fat composition is very different in artificially created formulas as well. The breastmilk itself contains factors that aid in digestion and absorption. The long chain fatty acids are required for adequate neurological growth[37]. These fats are precursors to the hormones required for infant growth. It appears that there is an unknown biological mechanism in mothers who deliver prematurely that increases the concentration of antimicrobial agents (sIgA, Lactoferrin and lysozyme), anti-inflammatory factors and immunomodulators. These immunological components are vitally important for the VLBW infant to prevent nosocomial infections, sepsis, necrotizing enterocolitis (NEC), bacterial and viral infections[8,9].

PRETERM ENTERAL NUTRITION

Well infants of gestational age > 34 weeks are usually able to coordinate sucking, swallowing, and breathing, and so establish breast or bottle feeding. In less mature infants, oral feeding may not be safe or possible because of neurological immaturity or respiratory compromise[1]. In these infants milk can be given as a continuous infusion or as an intermittent bolus through a fine feeding catheter passed via the nose or the mouth to the stomach. Human breast milk is the recommended form of enteral nutrition for preterm infants[1]. The milk could be from the infant's mother or expressed milk from donor mothers, who are usually mothers who have delivered term infants. The nutrient content of expressed breast milk varies depending on the stage of lactation at which it is collected. The following table shows the typical nutritional contents of human expressed milk[1].

TABLE

NUTRITIONAL CONTENT OF EXPRESSED BREAST MILK[1]

NUTRIENT	CONTENT / 100 ML
ENERGY	73 kcal
PROTEIN	2.7 g
FAT	3 g
CALCIUM	29 mg
PHOSPHATE	15 mg

The feeding method should be individualized based on gestational age, clinical conditions and the feeding tolerance of the infant[1]. Initially naso-gastric feeds are initiated in infants < 34 weeks as these infants have poor suck – swallow – breathe coordination patterns. Bolus feeds of milk is the method of choice that is currently being practiced. Boluses are given every 2 to 4 hours. The advancement of volume of feeds is carried out as follows in table.[1]

TABLE

TUBE FEEDING GUIDELINES[1]

Birth weight (grams)	Initial rate (ml/feed)	Volume increase (ml/feed)
< 800	10	10 – 20
800 – 1000	10 – 20	10 – 20
1001 – 1250	20	20 – 30
1251 – 1500	30	30
1501 – 1800	30 – 40	30 – 40
1801 – 2500	40	40 – 50
>2500	50	50

The goal of nutrition is to achieve as near to normal weight gain and growth as possible. It is difficult to deliver adequate calories with parenteral nutrition. So the aim should be to introduce enteral milk feeds as early as a baby can safely tolerate them. The calorie requirement of a preterm baby averages at about 120 kcal/kg/day. But this will vary between individual babies with some needing considerably more than this. The target weight gain when a baby is on full enteral feeds is between 10 and 25 grams/kg/day with an average of around 15g/kg/day. Weight gains in excess of 25g/kg/day should raise concerns about fluid retention. Babies less than 1750g at birth fed preterm compared to normal formula thrived significantly better; babies fed preterm formula compared to donor or maternal expressed breast milk also thrived better, babies fed maternal expressed breast milk had less NEC and better developmental quotients. More recent data from Schanler et al[11] have also suggested less sepsis and NEC in babies fed fortified breast milk. In light of the long-term health outcome advantages of expressed maternal breast milk together with probable short-term immunological advantages, this has to be the milk of choice [20]. The main debate on when to start feeds has centered on the potential increase risk of NEC with early enteral feeds. The evidence in this area is not strong. In the very preterm infant much of the recent focus has been on minimal enteric (trophic) feeding [20]**.** The principle behind this is to commence very low volume enteral feeds on day 1 to 3 of life. The term "trophic" refers to the animal data that suggested this approach augments the maturity of the gut both in terms of structure and function. A systematic review[11] suggests that Minimal enteric feeds allows earlier establishment of full enteral feeds and shorter hospital stays, without any concomitant increase in NEC. Well babies born after 32 weeks (33 weeks and over) can be started immediately on milk feeds. Well babies born between 29 and 32 weeks should have an IV inserted to allow slow increase in enteral feeds and milk started on day 1 at 1ml/hr [20]. Sick babies born after 28 weeks (29 weeks and more) should have enteral feeds commenced when it is felt clinically appropriate at 1ml/hr. Usually this would be day 2 or 3. Babies born before 29 weeks (28 weeks and less) should have minimal enteric feeds commenced at 1ml every 4 hours on day 2 or 3. In babies fed with milk from birth**:** Feeds should be increased as shown:Day 1- 60 mls/kg, Day 2- 90 mls/kg ,Day 3- 90 mls/kg ,Day 4- 120 mls/kg , Day 5- 120 mls/kg, Day 6- 120 mls/kg, Day 7- 150 mls/kg.[20]

BREAST MILK EXPRESSION / STORAGE

Mothers of premature infants who are being given tube feeds must pump their breast milk in order to establish and maintain an adequate supply of milk. An adequate volume of milk is defined as 18 to 20 oz over a 24 hr period at 2 weeks post- partum. The mother's milk supply is critical to successful nursing. If the baby is ill and feeding will be delayed for longer than 24 hours, mother should begin pumping her breasts immediately[22]. It is crucial to have the mother begin pumping within the first 24-48 hours after delivery to take advantage of the normal physiological changes after birth. If the breasts are not stimulated, prolactin levels will fall by 50% within the first five days after giving birth and will drop to non pregnant levels within seven days. The hospital should have several commercial hospital grade electric breast pumps available for these cases[16]. Small manual or electric pumps are not sufficient for establishing a milk supply. Instruct the mother to pump her breasts at regular intervals of every two or three hours around the clock, until the infant can nurse directly at the breast. This imitates, as closely as possible, what a normal breastfeeding pattern would be. Essentially, mother's body must be convinced that there is an infant to feed. Very early pumping is for stimulation purposes only[21]. It is important to tell the mother that no one expects her to obtain large volumes of milk initially. Babies can obtain colostrum easily from the breast because they strip the milk from the sinuses but colostrum does not pump out well. Most mothers obtain only a drop or two of colostrum for up to three days after birth. If she is told that there won't be much milk with the pump, she will be encouraged by any small amount produced [16]. What is important is the regular stimulation of the breasts to enhance the process of lactogenesis and to bring in a full milk supply. For the mother of the sick newborn, it is better to have mother over produce milk than to under produce. An abundant milk supply will assist in the baby's transition to breastfeedings[22]. The frequency and duration of milk expression directly correlates to the amount of milk produced. The mother should pump every two or three hours to mimic a baby's natural feeding pattern. This also takes advantage of the increased vascularity in the breasts and the high prolactin level following birth. Pumping at least once during the nighttime is vital in the first few weeks to maintain milk supply. The best results are obtained with double electric pump system because simultaneous breast stimulation elevates serum prolactin levels[16]. Continued use of a commercial, hospital grade electric pump increases compliance with the pumping schedule, and is better at maintaining milk supply. Mothers should pump as soon as possible after delivery. Mechanical expression needs to begin as soon as possible after giving birth, with the use of a hospital-grade electric breast pump[21]. Personal collection kits should be sterilized daily. Human milk expression in the hospital can take place at the infant's bedside or in designated private pump rooms. Mothers must be instructed in writing and/or verbally regarding appropriate pumping, labeling, storage, and transport technique. Human milk must be stored in "food-grade" plastic containers or glass. Human milk transported to and from the hospital should be maintained at proper temperatures (2°–6°C, 35°F–42°F), to prevent loss of nutrients and to minimize bacterial growth[1]. Dedicated freezers and refrigerators should be provided for storing human milk. Human milk should be stored in separate labeled bins or zippered bags, to prevent mis-administration of breast milk and to prevent cross-contamination of that milk with other feedings[16]. For proper breast milk storage, refrigerator temperatures should be maintained at 2°C to 4ºC (35°F to 40°F) and freezer temperatures at –20ºC (–4°F). Fresh human milk can be safely stored at 2°C to 4°C (35°F to 40°F) in the refrigerator for 48 hours. Infants should receive fresh breast milk whenever possible, because of the enhanced activity of cellular components[22].

CREAMATOCRIT

The creamatocrit is a simple, inexpensive, and accurate technique for estimating the lipid concentration and caloric density in mothers' milk. The creamatocrit technique is especially useful in the neonatal intensive care unit (NICU) to guide the fractionation and feeding of high-lipid, high-calorie hindmilk in extremely low birth weight infants when accelerated short-term weight gain is desirable[23]. The creamatocrit is a quick, accurate, easy-to-perform measure of the lipid content in mothers' milk. Used extensively in lactation research since 1978, it involves placing a few drops of milk in a capillary tube, and centrifuging it at 5000 rpm for 5 minutes so that the lipid or "cream" separates from the aqueous part of the milk[24]. Both the column of milk and the supernatant fat column are measured to the nearest 0.5 mm using a ruler[25]. Then, the percent of total milk volume equal to cream can be calculated and converted to estimates of lipid and caloric content using the formula:

$$\textbf{fat (g/l)} = \textbf{(creamatocrit \% - 0.59) / 0.146}$$

The creamatocrit is ideal to guide the separation of foremilk (low-lipid, low calorie milk) and hindmilk (high-lipid, high-calorie), when short-term accelerated weight gain in the infant is desired[26]. Because milk volume and rate of milk flow vary among women, a standardized set of instructions for separating foremilk and hindmilk will be ineffective. The creamatocrit can be used to teach mothers about their individual changes in milk lipid over the course of milk expression, and the women can be taught to provide milk with a targeted caloric content. In complicated clinical situations that require fluid restriction and concentrated calories, creamatocrits can be performed on each milk collection that is fed to an infant[26].

STUDY JUSTIFICATION

Following birth term infants rapidly adapt from a relatively constant intrauterine supply of nutrients to intermittent feedings of milk. Preterm infants, however are at increased risk of potential nutritional compromise. Infants less than 1.5 kg have a body composition of approximately 83 to 89% water, 9 to 15% protein and only 0.1 to 5% fat. Even this negligible amount of fat is mostly of structural origin and does not represent any calorific reserve[1]. Therefore caloric reserves are very minimal in the preterm infant. During the first several days of life, a substantial weight loss occurs both from tissue loss because of minimal caloric intake and from extracellular fluid loss. Even these meagre energy reserves of very low birth weight infants maybe depleted by 12 – 18 days of life even if given intravenous fluids supplying dextrose at the usual maintenance rates . The additional energy needed is primarily drawn from the catabolism of endogenous protein, probably skeletal muscle as opposed to fat stores[27]. Therefore insufficient exogenous protein and calories which is usually derived from the dietary fat, may prove to be life threatening to the sick, preterm infant. There has been conclusive proof that the nutrient intake during the first 6 weeks of life may have a significant impact on the development at 18 months of life and later[7]. Therefore the nutritional support of preterm and critically ill newborns is extremely important not only for survival but also for favorable long – term outcomes[6].

The rate of postnatal weight gain in human milk – fed, low birth weight preterm infants often does not match the rate in – utero. The goals of preterm nutrition according to currently accepted standards set by the American Academy of Pediatrics[1,20] is to limit the degree and duration of the initial weight loss that is seen over the first two weeks in preterm infants, to facilitate regain of weight gain within 7 to 14 days of life[1]. Replicating the growth of the fetus at the same gestational age, is also an appropriate goal.

These standards set for the growth of the preterm infants may be difficult to achieve, with human milk fed newborns. This may be due to a variety of factors. For example, milk intake maybe limited by the fluid volume restrictions and thus nutrient intake maybe insufficient[1]. Despite reportedly greater quantities of protein in preterm than full term milk, the content of protein, sodium, and trace elements decreases during the course of lactation[22]. Increasing fluid administration and fortifying human milk do not compensate for the marked variability in the lipid composition of human milk [25, 22]. This fat content is known to increase during a single feeding from foremilk to hindmilk, and from morning to night. In addition there is significant inter - individual variation[27]. These factors are clinically important as nearly 50 % of the energy content of human milk is derived from fat[24]. When exogenous lipid sources are added as energy supplements to human milk, they are poorly miscible, separate from the milk and adhere to feeding tubes and collection vessels[42].

There have a been few studies in the past that have tried to use the known variability in the human milk lipid content to improve the weight gain rate of newborns[15], especially preterm newborns. The use of hindmilk rich completely expressed human milk increases the endogenous lipid content of human milk. This can improve the rate of weight gain of newborns. In order to test this hypothesis which has been validated only in some small size samples so far, this study was conducted in this neonatal medical unit.

LITERATURE REVIEW

1. **Promoting the exclusive feeding of own mother's milk through the use of hindmilk for hospitalized preterm infants- Slusher et al, J Hum Lact 19(2) 2003.**

   - This study was used to determine if hindmilk feedings of own other's milk as reported in the united states could be instituted in the NICU to improve weight gain of preterm infants.
   - A group of 16 preterm infants were initially being given foremilk feeds. These infants had a poor weight gain and they were then switched over to exclusive hind milk feeds.
   - It was proved that in the hindmilk intervention period these infants recorded a significant increase in the weight gain (18.8g/d) compared to 8.6 g/d during the foremilk feed period. But the small sample size and the lack of a control group were some major drawbacks in this study.

2. **Hindmilk improves weight gain in LBW infants fed human milk – Valentine et al, Journal of Pediatric Gastroenterology and Nutrition 18:474 – 477, 1994**

   - This study was conducted in a tertiary care newborn nursery in the United states.
   - 15 low birth weight infants were included in the study, and given completely expressed hindmilk feeds , after an initial period of slow eight gain was documented in these infants during the period of use of conventionally expressed milk feeds.

- There was a significant difference between the fat content of the milk used in the study group which had a higher fat content than the milk used in the control group.
- There was no significant difference in the contents of other nutrients like, Nitrogen, Calcium, Copper, Potassium and Sodium, between the milks used in the two groups.
- The rate of weight gain during and after the period of hindmilk intervention was significantly higher, than the weight gain noted in the period when conventionally expressed milk was used. The corresponding weight gain values were 15.5 gm/kg/d and 6.2 gm/kg/day
- This study too has been hampered by the small sample size, and the lack of a separate control group. The authors recommend that larger studies are needed to analyze the effect of hindmilk use further.

3. **Individualizing the lipid content of Own Mother's Milk: effect of weight gain for the Extremely Low birth weight Infants. Meier et al, Pediatr Res 1998; 43: 270A**

- This study conducted in a tertiary care nursery in the United States. Its aim was to measure and individualize the fat content of mother's expressed milk used to feed the preterm ELBW infants and to use only the milk with higher fat content to feed them.
- The study consisted of 64 newborns who were given this fat content rich completely expressed milk over a period of 2 to 4 weeks. Their weight gain during this period was computed and then compared with the average weight gain of newborns of a similar group treated in the

nursery, but given conventionally followed milk feeds.

- Fat content was measured using the creamatocrit technique.
- This study showed that the fat content as assessed by creamatocrit technique correlated well with the actual fat content of milk
- It also showed that the preterms had a higher weight gain following the period of the use of high fat content milk. This weight gain increase was statistically significant when compared to the earlier weight gain rates achieved by these neonates when on conventional milk feeds.
- This study had the drawback of using a retrospective - prospective model rather than completely prospective study model which is the design of choice when evaluating a new mode of intervention.

4. **Creamatocrit: simple method for estimating the fat concentration and energy value of human milk, Lucas et al, British Medical Journal. 1978;1:1018 – 1020**

- This was the first study that proved and advocated the use of the Creamatocrit in measuring the fat content of expressed human milk.
- The study compared the fat content of a large number of expressed milk samples obtained from lactating mothers belonging to a wide variety of social, economic, and cultural strata. Fat content was measured using both the new creamatocrit technique and the standard gravimetric method of fat estimation.
- There was a good correlation (Kappa values of > 0.8) between both these methods, which was very significant.

5. **Feeding Strategies for Premature Infants: Randomized Trial of Tube-feeding Method, Schanler et al, Pediatrics vol. 103 no. 2 February 1999, pp. 434-439.**

- This study was a meta analysis of the use of human milk and various preterm feeding protocols that were being followed in newborn nurseries across the United states between 2002 – 2004
- After analysis of an extensive variety of feeding protocols with respect to the type of feeds used, the initiation and step up pattern of feeds etc., the authors have concluded that goal oriented nutritional programs, that were aimed at early initiation of enteral feeds and monitoring of the energy content of milk used, were very important to help the preterm newborn to achieve a satisfactory growth rate.

6. **Manual and pump methods of expression of breast milk, Taneja U, Indian J Pediatr. 1996 Jan-Feb;63(1):87-92.**

- This was a study conducted in AIIMS, New Delhi
- It involved a comparison of hand expression of milk vs pump expression of milk for mothers of preterm infants.
- The authors of this study have opined that in most of our newborn nurseries, and in most other developing nations, milk is routinely hand expressed at regular intervals just prior to a low birth weight infant's feeding time. Breast pump expression of milk, or even complete hand expression of milk is not followed in our nurseries.
- The use of milk obtained in this way leads to use of predominant foremilk feeds for the preterm newborns, and also leads to reduction of

milk volumes for the lactating mothers as they fail to promote the let down reflexes at this sensitive early period of lactation

7. **Weight gain in exclusively breast fed preterm infants, Ramasethu et al,J Trop Pediatr. 1993; 39: 152 – 159.**

- This study was conducted in Tamilnadu.
- The authors performed a review of the method of preterm feeding being followed in about 12 newborn nurseries in the state.
- They analyzed the effect of these preterm feeding protocols on the growth of these neonates.
- The authors concluded that the only feasible method of preterm feeding in our settings was exclusive breast feeds. But they also noted that existing feeding methods and regimens were not proving to be effective in achieving desired rates of preterm growth. They recommended that newer methods and regimens needed to be tried in improving this area of preterm care.
- They suggest that use of hindmilk feeds or completely expressed milk could be a solution that could prove viable in the long term

8. **Adherence of medium-chain fatty acids to feeding tubes during gavage feeding of human milk fortified with medium-chain triglycerides. Mehta et al, J Pediatr. 1988 Mar;112(3):474-6**

- This study was aimed at estimating the amount of exogenous fat that was actually bioavailable to the preterm gavage fed newborns.
- The data show that only trace amounts of lipid (0.23 +/- 0.04%) adhered to feeding sets during feeding of unfortified human milk.

- Significantly more lipid (p less than 0.0005) adhered when human milk was fortified with MCT oil, and the method of feeding greatly affected lipid adherence.
- This shows that such methods of exogenous fortification of human preterm milk fat content are not always useful.

9. Early diet in preterm babies and developmental status in infancy, A Lucas et al, Arch Dis Child 64: 1570, 1989.

- Few data from randomized prospective studies address whether early diet influences later neurodevelopment in man.
- As part of a larger multicentre trial, 502 low birth weight infants were assigned randomly, for a median of 30 days, to receive a preterm formula or unfortified donor breast milk as sole diets or as supplements to their mothers' expressed milk. Surviving infants were assessed at nine months after their expected date of delivery without knowledge of their feeding regimen.
- The mean developmental quotient was 0.25 standard deviations lower in those fed donor breast milk rather than preterm formula.
- The authors suggest that the diet used for low birth weight babies over a brief, but perhaps critical, postnatal period has developmental consequences that persist into infancy.
- Infants who are small for gestational age are especially vulnerable to suboptimal postnatal nutrition.

10. Macronutrient and energy contents of human milk fractions during the first six months of lactation, Sareela t et al, Acta Pediatr 2005 Sep;94(9):1176-81.

- To study the macronutrient and energy contents of human milk fractions during the first 6 mo of lactation.
- The protein content was significantly lower in fore- and hindmilk than in donor or preterm milk during the first months of lactation. Lactose content showed no significant changes between the groups or in the course of lactation. The fat content was highest in hindmilk, being approximately two- to threefold that of foremilk. Accordingly, hindmilk included 25-35 kcal/100 ml more energy on average than foremilk.
- The fat content of human milk increases in relation to breast emptying, while the other macronutritients of milk show only slight changes.
- When enteral feeding with high-energy human milk is preferred, as in the case of very preterm infants, hind milk, with its higher fat content, would be a natural choice.

AIM OF THE STUDY

PRIMARY OBJECTIVE

To study the effect of completely expressed hindmilk rich feeds vs conventionally expressed breastmilk feeds in increasing the weight gain of preterm newborns

SECONDARY OBJECTIVE

To study the difference in the fat content of completely expressed hindmilk rich feeds vs conventionally used hand expressed milk feeds and to correlate this to the difference in weight gain, if any.

MATERIALS AND METHODS

STUDY PLACE :

Neonatal unit, Institute of Child Health and Hospital for Children, Chennai

STUDY PERIOD:

January 2004 – January 2006

STUDY DESIGN:

Randomized Controlled Clinical Trial

STUDY POPULATION:

Preterm newborns of gestational age 28 – 32 weeks, admitted to the neonatal unit for preterm care and without complications of prematurity

INCLUSION CRITERIA:

Preterm newborns, 28 – 32 weeks of gestational age, admitted to the newborn ward for preterm care and on expressed milk feeds by nasogastric tubes or paaladai feeds

EXCLUSION CRITERIA:

Preterm newborns with other associated complicating illnesses that might affect weight gain such as PDA, NNEC, IVH, sepsis etc were excluded from the study even if they satisfied the above inclusion criteria.

Also, all term newborns were excluded from the study as the study as restricted to the use of expressed milk in preterm neonates

SAMPLE SIZE:

The sample size was calculated using an α error of 0.05, and a power of the study at 80% and with a previous similar study by Valentine et al showing a difference of 7 mg/kg/day with a between - subject standard deviation of 4.4. The sample size calculated with these requirements to give a meaningful result was 69 patients in both the groups.

STUDY MANOEUVRE

Preterm newborns admitted to the neonatal unit of the Institute of Child Health and Hospital for Children, between the gestational ages 28 and 32 weeks, who were admitted for preterm care and started on expressed milk feeds either by nasogastric tubes or by paaladai, were included in the study. An informed consent was obtained from mothers of all the newborns enrolled in the study. Approval for the study had been received from our institutional review board.

Those preterm newborns who fulfilled the inclusion criteria but also had other intercurrent illnesses that might have an effect on the nutrition and weight gain of the newborns were excluded from the study. The postnatal age at the time of inclusion in the study, the birth weight, the gestational age as assessed by the New Ballard's score (Appendix 2) were recorded. Classification into SGA/AGA/LGA according to birth weight and GA using the Lubchenko's intrauterine growth curves (Appendix 3) was also done and recorded for all the newborns at the time of inclusion into the study.

Following inclusion into the study, all newborns were randomized into two groups using block randomization method using a standard table of random numbers, by an independent observer who was blinded as to the purpose or the need for the randomization.

All the newborns involved in the study were given expressed, own mother's, unfortified milk either by a nasogastric tube or by a paaladai depending upon the level of maturation of their suck – swallow coordination which varied with their gestational age. The newborns in the control arm were given milk obtained by the currently followed method in our nursery which consists of hand expression of just the desired volume of milk at regular interval just prior to the newborn's scheduled feeding times. The mothers in this group were asked to express milk as they had been practicing from their first day of stay in the nursery.

The newborn's in the study arm were given expressed milk obtained by complete expression of the breasts by means of a breast pump expression using Medela, single collection set, electric breast pumps. The milk thus expressed was stored in glass containers in a refrigerator in the unit at 4 – 5 ° c at which temperatures, milk remains stable and free of contamination for at least 48 hours. Milk so obtained was used for the babies in the study arm. Complete expression was accepted as cessation of milk flow at the end of every expression procedure, in order to ensure optimum milk removal.

Both groups were fed using the existing unit feeding protocol which is based on well established and followed guidelines for preterm feeding. Feed initiation and advancement was carried out in accord with this protocol which was adhered to in both the groups without any difference.

The daily feed volume and the average feed volume for every newborn under the study was recorded. The number of days the newborns remained in the study protocol was also recorded.

The mothers in the control arm were asked to provide twice daily samples of their expressed milk obtained between 6 to 9 AM and later from 9 PM to 12 midnight in order to analyze fat content by the creamatocrit method. Samples of the completely expressed milk from mothers in the study group were also obtained at similar schedules. Twice daily samples were collected in order to overcome the sampling errors due to the known diurnal variation of the milk fat content.

Fat content was analyzed by creamatocrit as follows. About 75 μL aliquots of the milk samples from mothers were placed into glass capillary tubes sealed at one end and centrifuged for 5 minutes at 9000 rpm. During centrifugation the fat present in the milk separates from the main column of milk and becomes a supernatant fraction of the main column. The length of the total milk column and the length of the supernatant fat column were measured to the nearest 0.5 mm using an ordinary ruler. The creamatocrit value was expressed as a percentage of the fat column length to that of that of the total column of milk. Each reading was provided in a blinded manner by an independent investigator, who was not aware of the origin or purpose of the measurements being done. These measurements were carried out daily in the milk used to feed the newborns in the study. Finally, these daily values were used to obtain the average fat content of the milk used to feed each newborn.

Nude daily weight of all the newborns in the study was measured using a digital Baby weighing electronic scale, accurate to the nearest 0.5 gm. Weight measurement was carried out at a fixed time each day to minimize errors. At the end of the expressed milk feeding regimen, the final weights of all newborns were recorded. The difference between the initial and final weight of every newborn was obtained. This value was divided by the average weight of the newborn during the period under study, to calculate the weight gain in gm/kg/day units.

In the event of newborns included in the study developing complications such as sepsis, NNEC, feed intolerance, apnoea of prematurity or other illnesses that warranted discontinuation of enteral feeds, then those babies were excluded from the study. Those newborns that entered the study and were fed according to the specified tube feeding protocol for a minimum period of at least 12 days, (defined so in order to overcome the period of initial weight loss which might ensue in the immediate postnatal period), were defined to have completed the study, and their data was included in the final analysis of the results. The observations from the study, primarily the fat content of the milk and the weight gain of the newborns, were analyzed for statistical significance as described below.

STATISTICAL ANALYSIS

1. Descriptive statistics of various factors analyzed in the study such as gestational age, birth weight, initial weight, sex ratio, weight – GA relationship categories, number of days in the trial, volume of feeds, creamatocrit values and weight gain. Means and standard deviations were used for the interval data and frequencies were used for ordinal data.
2. Student's t test analysis to investigate the statistical significance of the difference between the mean weight gains of the two groups
3. Student's t test to analyze the statistical significance of the difference between the mean fat content of the two groups
4. Spearman's rank correlation coefficient between the fat content of milk and the weight gain of each newborn.
5. Linear regression analysis to assess the relationship between the weight gain and the milk fat content.

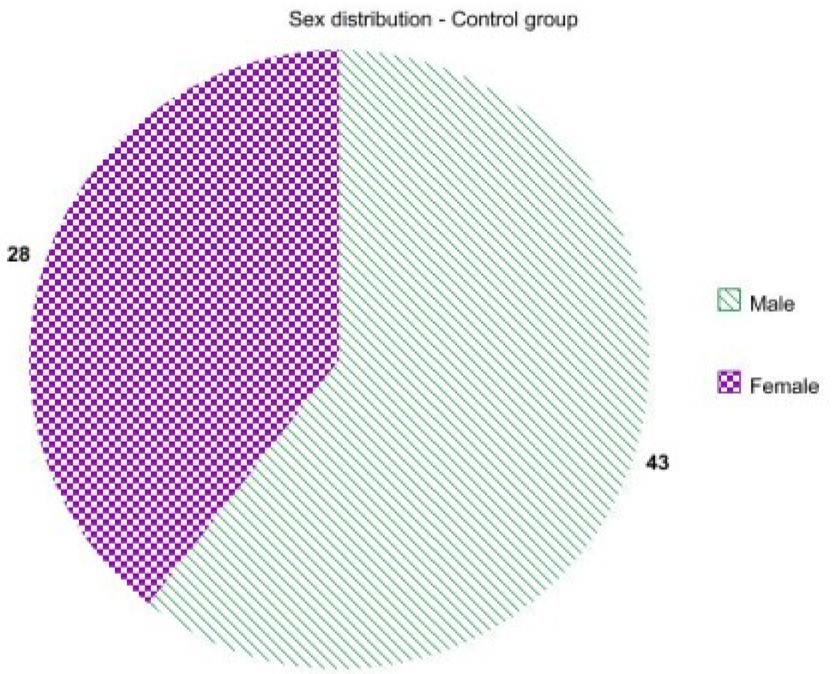
OBSERVATIONS

A total of 172 cases fulfilled the inclusion criteria and were enrolled in the study. Of these, 28 cases were further excluded from the study at various points of time. Out of these 28, 16 cases developed illnesses that precluded enteral feeding (7 cases of sepsis in the control group, 5 cases in the study group, 2 cases with NEC in each of the two groups). 12 cases did not complete the required number of days (> 12) of expressed feeds and were able to feed directly at the breast (6 cases in each of the two groups). So the data from the remaining 144 cases was taken up for statistical analysis. The technique of block randomization ensured that the study group as well as the control group had a similar number of subjects – 86 for each group, to begin with. Owing to the above reasons the final number of newborns remaining in the study group at the end of the study was 73. The control group had 71 newborns at the end of the study.

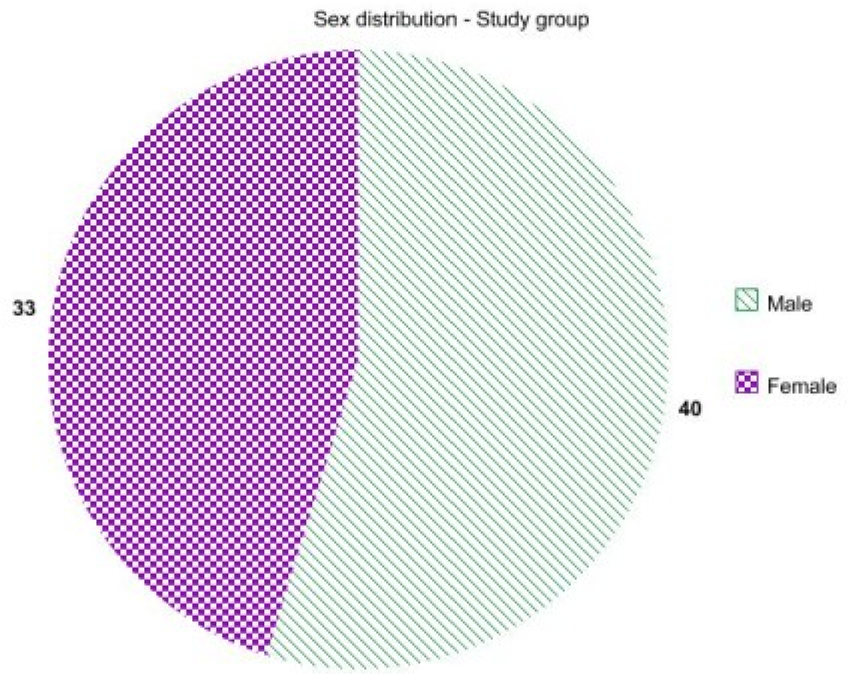
Further results are displayed in the form of tables and diagrams as follows.

FIGURE 1

COMPARISON OF SEX DISTRIBUTION



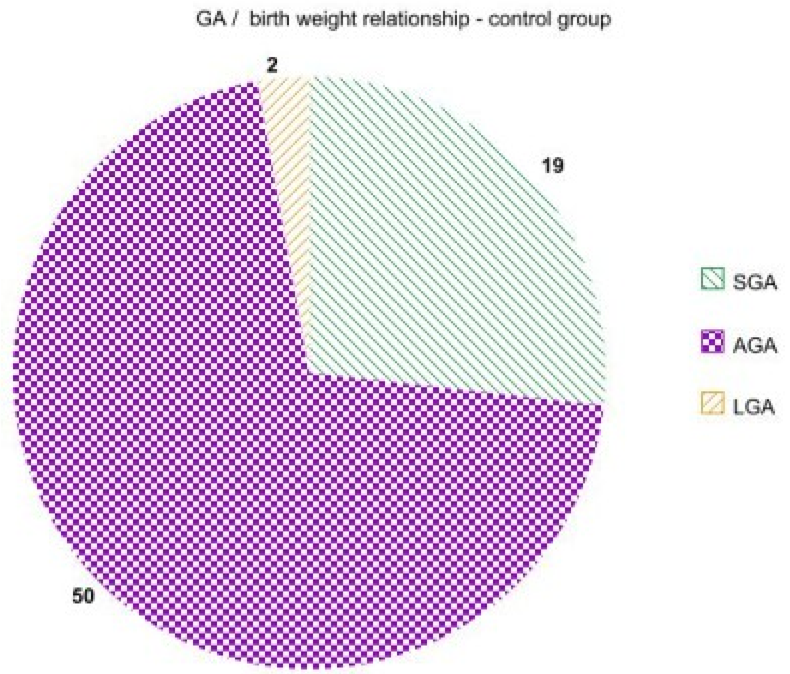
The Male : Female ratio for the CONTROL group is 1.6:1 and for the STUDY



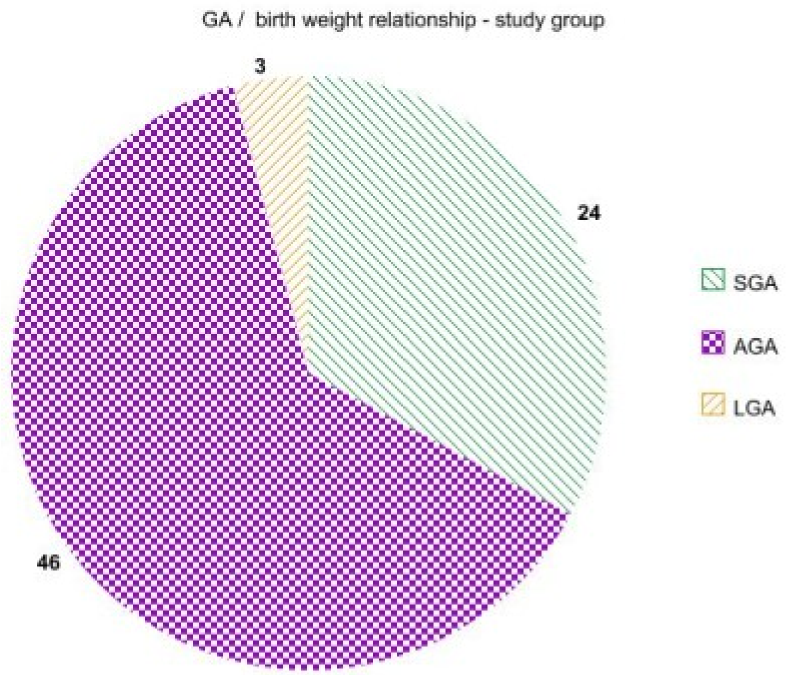
group it is 1.3:1

FIGURE 2

COMPARISON OF GESTATIONAL AGE – BIRTH WEIGHT RELATIONSHIPS



There was no significant difference between the 2 groups regarding this



distribution.

TABLE 1

COMPARISON OF AVERAGE BIRTH WEIGHT

BIRTH WEIGHT in kgs	STUDY GROUP	CONTROL GROUP
MEAN	**1.648**	**1.657**
VARIANCE	0.06	0.07
STANDARD DEVIATION	0.25	0.26
STANDARD ERROR	0.02	0.03
COEFFT. OF VARIANCE	15%	16%

't' statistic : - 0.24,   p value: 0.8102.

There is no statistically significant difference between the average birth weights of the newborns in the 2 groups.

TABLE 2

COMPARISON OF AVERAGE INITIAL WEIGHT (WEIGHT ON DAY 1 OF STUDY PERIOD)

INITIAL WEIGHT in kgs	STUDY GROUP	CONTROL GROUP
MEAN	**1.624**	**1.630**
VARIANCE	0.06	0.06
STANDARD DEVIATION	0.24	0.25
STD ERROR OF MEAN	0.02	0.03
COEFFT. OF VARIANCE	15%	16%

't' statistic: -0.14   p value: 0.88

There is no statistically significant difference between the average initial weights of the newborns in the 2 groups.

TABLE 3

COMPARISON OF AVERAGE NUMBER OF DAYS IN TRIAL

NUMBER OF DAYS IN TRIAL	STUDY GROUP	CONTROL GROUP
MEAN	**13.9**	**14.9**
VARIANCE	1.71	1.08
STANDARD DEVIATION	1.30	1.04
STD ERROR OF MEAN	0.15	0.12
COEFFT. OF VARIANCE	9%	7%

't' statistic : -0.07 two tailed p value : 0.94

There is no statistically significant difference between the average number of days spent in the trial for the newborns in the 2 groups.

TABLE 4

COMPARISON OF AVERAGE FEED VOLUME

FEED VOLUME in ml/day	STUDY GROUP	CONTROL GROUP
MEAN	**123.34**	**127.32**
VARIANCE	111.72	121.90
STANDARD DEVIATION	11.47	11.48
STD ERROR OF MEAN	1.35	1.35
COEFFT. OF VARIANCE	7%	8%

't' statistic: 1.0 p value: 0.306

There is no statistically significant difference between the average feed volumes for the newborns in the 2 groups.

TABLE 5

COMPARISON OF AVERAGE FINAL WEIGHT

FINAL WEIGHT	STUDY GROUP	CONTROL GROUP
MEAN	**2.068**	**1.806**
VARIANCE	0.08	0.05
STANDARD DEVIATION	0.28	0.24
STD ERROR OF MEAN	0.03	0.02
COEFFT. OF VARIANCE	14%	13%

't' statistic: 6.42 **p value: <0.001**

There is a HIGHLY <u>statistically significant difference</u> between the average FINAL WEIGHTS at the end of the trial for the newborns in the 2 groups.

TABLE 6

COMPARISON OF RATE OF WEIGHT GAIN

RATE OF WEIGHT GAIN mg/kg/day	STUDY GROUP	CONTROL GROUP
MEAN	**19.58**	**8.895**
VARIANCE	6.34	8.41
STANDARD DEVIATION	2.51	2.90
STD ERROR OF MEAN	0.29	0.34
COEFFT. OF VARIANCE	13%	37%

't' statistic: 24.3 **p value: 0.001**

There is a HIGHLY statistically significant difference between the average RATES OF WEIGHT GAIN for the newborns in the 2 groups.

TABLE 7

COMPARISON OF AVERAGE FAT CONTENT OF MILK

FAT CONTENT OF MILK in CRCT %	STUDY GROUP	CONTROL GROUP
MEAN	**8.557**	**5.413**
VARIANCE	1.97	2.28
STANDARD DEVIATION	1.40	1.51
STD ERROR OF MEAN	0.16	0.17
COEFFT. OF VARIANCE	16%	28%

't' statistic: 12.78 **p value: < 0.001**

There is a HIGHLY statistically significant difference between the average FAT CONTENT OF MILK for the newborns in the 2 groups.

TABLE 8

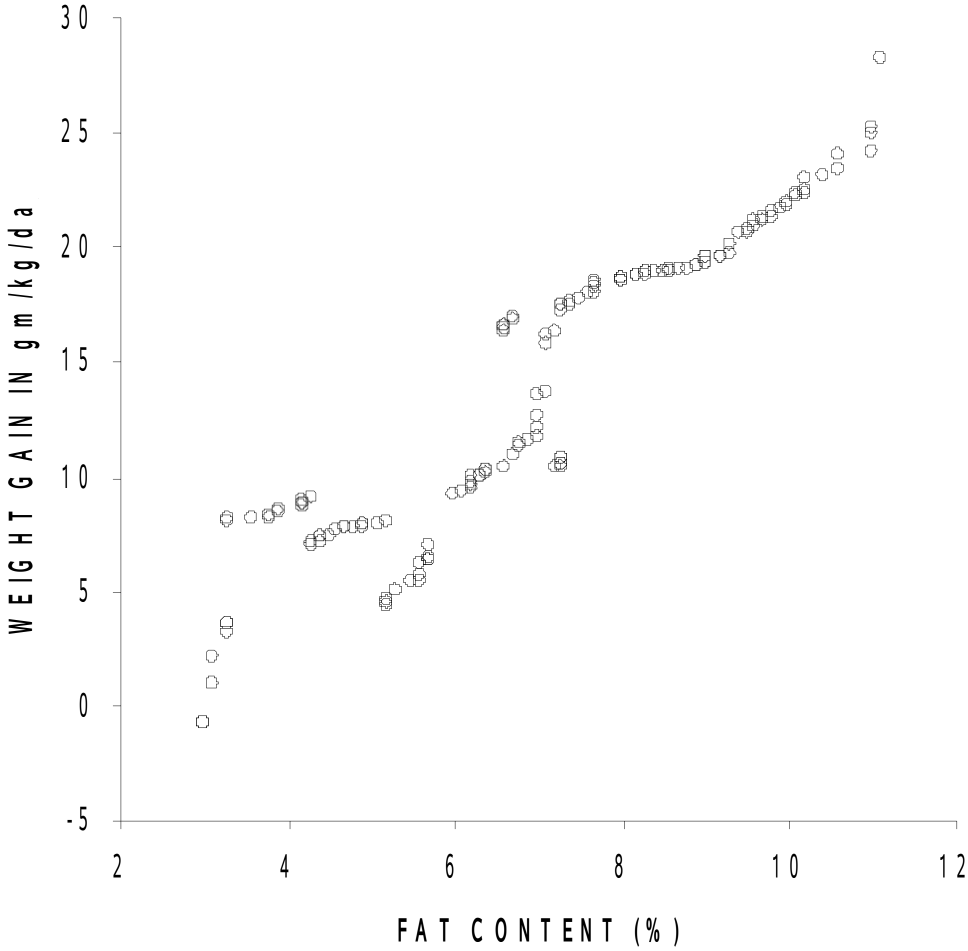
COMPARISON OF WEIGHT GAIN RATES ACROSS DIFFERENT SUBSTRATA

STRATA	STUDY GROUP	CONTROL GROUP	P Value
SGA	15.62 ± 2.51	5.44 ± 1.99	**<0.01**
AGA	18.80 ± 3.20	8.02 ± 2.68	**<0.01**
LGA	21.56 ± 3.00	9.98 ± 2.98	**<0.01**
GA- 28 – 30 WEEKS	20.36 ± 2.91	8.86 ± 2.46	**<0.01**
GA- 30 – 32 WEEKS	15.50 ± 2.00	6.98 ± 2.30	**<0.01**

In all these substrata there is **a significant difference in the weight gain rates between the study group and the control group,** with the **study** group showing a consistently **HIGH** rate of weight gain across all groups.

FIGURE 3

CORRELATION BETWEEN THE FAT CONTENT OF MILK AND THE WEIGHTGAIN OF THE NEWBORNS



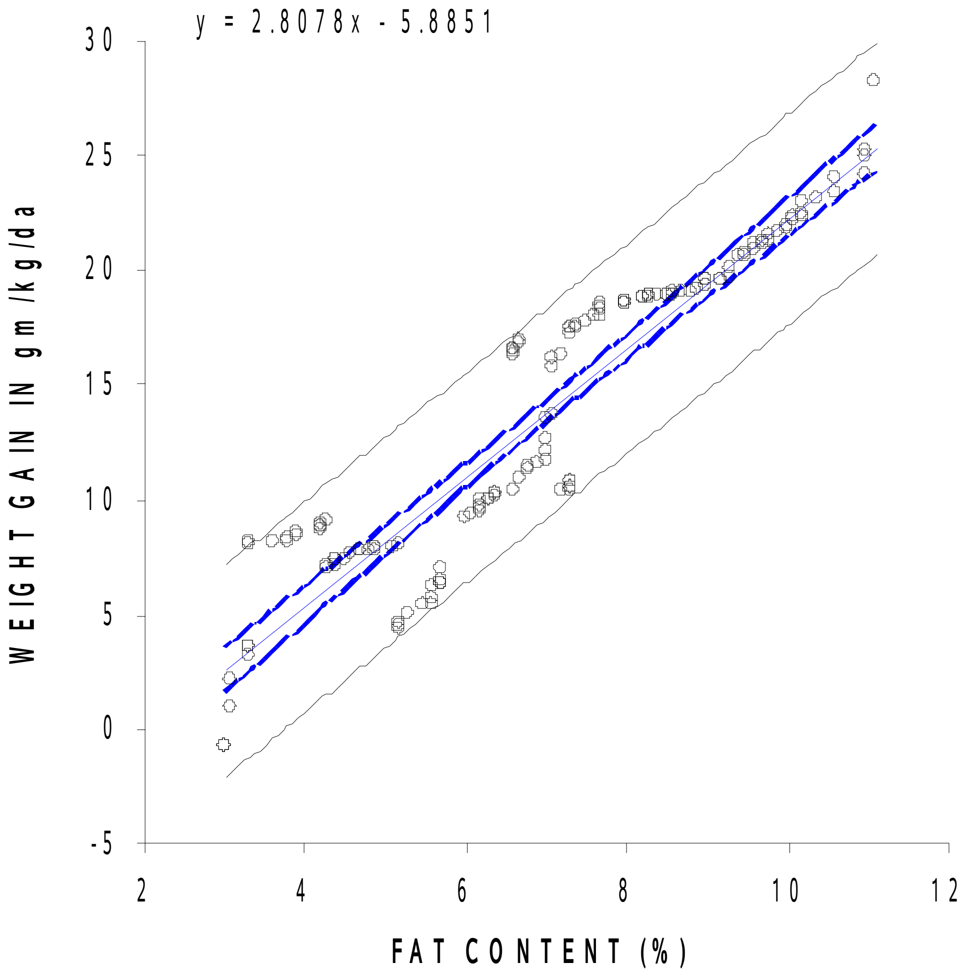
Spearman's rank Correlation coefficient : 0.88

There was a statistically significant correlation between the weight gain and the fat content of the milk.

FIGURE 4

LINEAR REGRESSION ANALYSIS BETWEEN FAT CONTENT AND

WEIGHT GAIN



The regression curves are linear and the regression equation shows the relationship between the fat content of milk and the weight gain of the newborns.

DISCUSSION

This study was a clinical trial to compare the effectiveness of using predominantly hindmilk feeds to increase the rate of growth of the preterm newborn. One group of newborns was given completely expressed milk feeds which was predominantly fat rich hindmilk and another group of newborns was given the usually followed method of partially expressed predominantly foremilk feeds. The aim of the study was to show that this method of incomplete expression produces milk with a poor fat content and causes a less than desired rate of weight gain for the preterm breastmilk- fed preterm newborn. The data was analyzed descriptively and for statistical significance between the outcome measures of the two groups.

The Male : Female ratio for the study group is 1.3:1 and for the control group it is 1.6:1. These values are quite similar to each other. Thus gender distribution differences were not observed between the two groups. There has been no consistent effect of gender differences on the weight gain of newborns in the early newborn period as earlier studies have shown[19,21]. There was also no significant difference in the weight gain between male and female newborns of both the groups.

Its well known that the birth weight of the newborn has a significant impact on the rate of post – natal weight gain[19,43]. It has been shown in previous studies that the rate of growth, especially weight gain, is higher for newborns with higher birth weights[43]. In this study, the mean birth weight of the preterm newborns enrolled in the control group was 1.648 ± 0.25 kg for the study group while that for the control group was 1.657 ± 0.26 kgs. The highest and lowest values for the study group were 1.22 and 2.26 kgs. Similar values for the control group were 1.28 and 2.30 kgs. There was no statistically significant difference between these measures. Thus a difference in birth weight could not have affected the final outcomes.

The average postnatal age of the newborns at the time of enrollment in the study was in the study group was 7 ± 1.2 days whereas the average value for the control group was similar at 8 ± 1.3 days. There was significant difference between the two groups regarding this variable. Another weight related variable that could have affected the final outcome of the study is the initial weights of the newborns at the point of enrollment into the study. As can be expected from the similarity between the two groups in the average birth weight and the average postnatal age at the time of enrollment in the study, the average initial weight of the newborns was also similar. The study group had an average weight of 1.624 ± 0.24 kgs while the control group had a value of 1.630 ± 0.25 kgs. There was no statistically significant difference between the two groups regarding this variable.

The frequency distribution of the gestational age groups for the study group was, 28 in the 28 to 30 weeks category and 44 in the 30 to 32 category. There were no subjects below or above these age groups as the study was restricted to tube fed or paaladai fed newborns who usually fall into the above two GA groups. The similar distribution for the control group was 32 and 40 respectively. The relationship between the birth weight of a newborn and the gestational age of the newborn is a measure of its postnatal growth potential. It has been shown in previous studies by Hediger et al[44] that among the three groups LGA babies have a significantly higher rate of postnatal weight gain, followed by AGA babies while SGA babies tend to grow the slowest of all. The distribution of the newborns in the three categories of Appropriate for gestational age (AGA), Small for gestational age (SGA), and Large for gestational age (LGA), was another confounding factor of the final outcome that was also analyzed for difference between the two groups. The distribution for the study group was AGA – 46, SGA – 24, LGA – 03. The distribution for the control group was AGA – 50, SGA – 19, LGA – 02. There was no significant difference in the frequency distribution of these variables between the two groups. As is obvious, any difference in the average feed volume between the two groups could have had a significant impact on the rate of weight gain. This confounding factor was also analyzed.

The average feed volume for the infants in the study group was 123.32 ml/kg/day while that for the control group was 127.34 ml/kg/day. There was no statistically significant difference between the two groups. Similarly the average duration of stay in the study was 13.9 ± 1.3 days for the study group and 14.9 ± 1.04 days for the control group. There was no difference in these aspects as well between the two groups.

Analysis of the fat content of the milk used in the study group showed that the average content of the fat in the milk used to feed these newborns who were given completely expressed (hindmilk-rich) feeds was 8.56 ± 1.40 % as measured by the creamatocrit. The corresponding value for the control group newborns who were given the currently followed nursery practice of hand expressed low volume expression feeds was 5.41 ± 1.51. The average fat content of breast milk has been estimated to vary between 6 to 8%. Thus while the fat content of the completely expressed milk was higher than average, the fat content of the milk used in the control group which is only partially expressed has been shown to be in the lower ranges of the normal values. There was a significant difference between the two groups regarding this variable. Similar studies by Schanler et al[14] have shown that hindmilk rich milk has a fat content of 8 to 10%.

The outcome measured in the study was the final rate of weight gain of the newborns in the two groups. The study group newborns had an average rate of weight gain of 19.58 2.51 gm/kg/day. The control group had a weight gain rate of 7.895 gm/kg/day. This difference was statistically significant at p values of < 0.001, which is highly significant statistically. Thus there was a definite difference in the rates of weight gain and the study group of completely expressed hindmilk rich feeds demonstrated a higher weight gain.

Thus of the various confounding factors, that could have had an effect on the outcome (the rate of weight gain), most factors such as sex ratio, gestational age differences, inherent growth potential differences, and factors brought into play by the study manouevres, such as the duration of stay in the study, and the feed volumes were found to be equally distributed between the two groups. There is however a significant difference between the groups with regard to the fat content of the milk used between the two groups. This difference has been shown to correlate highly with the difference in the rate of weight gain observed between the two groups (Spearman's rank Correlation coefficient : 0.88) . On linear regression analysis a straight line positive relationship was obtained between the fat content and the rate of weight gain for both groups of newborns. As fat content of the milk increases the rate of weight gain of the newborns also shows an increase. This effect has been observed even for the small increments in the fat content of the milk observed in the control group and more so for the larger increments in the fat content observed in the study group.

Some interesting issues aside from the main aim of the issue were brought to light in this study. The significant difference in the rate of weight gain between the two groups also held good, across the various subgroups analyzed in the study. The rate of weight gain was highest in the AGA group. This difference was observed in both the groups. This has been well established in some previous studies[44]. Similarly rates of weight gain were lowest for the SGA category in both the groups. Another issue was the rate of weight gain across different GA groups. It was observed that the highest rate of weight gain was observed for the newborns in the 28 to 30 weeks of GA category, in both the groups. This probably reflects the fact that the rate of weight gain in utero follows sigmoidal patterns and thus the tapering of the weight gain observed during the last trimester of gestation has also been replicated in the post natal weight gain of these infants. The desired rates of post natal weight gain for infants of different GA groups have been proposed by various authors such as Babson and Brenda et al, Nelson et al, and Shaffer et al[1]. The table below shows some of these recommendations. The curves are given in the appendix 3.

GESTATIONAL AGE AT BIRTH (wks)	DESIRED RATES OF POST NATAL WEIGHT GAIN (mg/kg/day)
24 – 28	15 – 20
29 - 32	17 - 21
33 - 36	14 - 15
37 - 40	7 - 9

It can be seen from the above data that the desired rates of weight gain vary inversely with the gestational age of the newborn. Thus preterm newborns in the lower range of gestational age are also the group in which the highest rate of weight gain is desired. There is a striking discrepancy between the weight gain observed in the control group when compared to the desired rates of weight gain depicted in the above table. It is also observed that the study group of newborns have achieved the desired rates of weight gain across all subgroups of gestational ages. This shows that this method of feeding of newborns with completely expressed hindmilk rich feeds or hindmilk fractionated feeds is an effective way of achieving the desired rates of weight gain for preterm infants.

There have been few similar studies in the literature that have attempted to perform a similar analysis of the effect of completely expressed or predominantly hindmilk feeds in improving the growth rate of preterm newborns. Some of these studies and their results have shown that there is a similarity between the present study and the studies in the past. These studies have been mentioned in the literature review section with their full citations.

A study by Slusher et al (2002) in Nigeria, was done as a cross-over study to compare the effect of completely expressed milk vs predominant foremilk feeds in increasing the weight gain rate of preterm newborns. The mean difference in weight gain rate in this study was 8.7 gm/kg/day. A similar study was conducted in the United states, by Valentine et al (1994), which also was a crossover study of 16 newborns comparing the influence of hindmilk vs foremilk feeds in increasing the weight gain rate of preterm newborns. The rate of weight gain in the period of hindmilk feeds was 15.5 gm/kg/d and 6.2 gm/kg/day. To our knowledge there have been no previous studies in the literature that have been done as a prospective randomized trial to study this hypothesis. This study used such a design to study the effect of hindmilk and as the results discussed indicate, the growth rate of preterm infants can indeed be increased by such interventions.

In the developing world,milk is routinely hand expressed at regular intervals just prior to an LBW infant's scheduled feeding times. Several reports indicate that hand expression is associated with lower milk volumes than manual or electric breast pump

Our observations of hand expression practices use in the nursery where this evaluation was conducted suggest that low milk volume may be a result of incomplete breast emptying overtime, in that mothers often removed only the approximate amount of milk that their LBW infants required at each feeding. Because infants are small, this volume maybe only 20 to 30 mL of milk. This incomplete milk removal, especially during the early days and weeks of lactation, triggers a cascade of local and central inhibitory responses that compromises prolactin release and milk yield.

In the last few years, the nutritional benefits of human milk for the preterm infant, in terms of protein digestion, fat absorption, lactose digestion and amino and fatty acid patterns have been increasingly recognized even in developed countries. Human milk provides unique protection from such diseases as septicemia and necrotizing enterocolitis which is especially important in high-risk infants. The lipids in human milk make up 50% of the calorie content and are structured in a way that promotes digestion and absorption--an ideal combination for an infant that needs to gain weight. However, since the amount of fat in human milk varies between individual mothers and from feeding to feeding, a strategy is needed to ensure that milk with the highest fat content is fed to the infant. Such lacto-engineering strategies as the method described in this study that use predominantly hindmilk feeds to feed the infants are successful in increasing the body weight gain rates of the preterm infant to the desired levels and help in optimizing preterm growth. This strategy which is cost -effective, safe and hygienic, is of special importance regarding its applicability in resource poor settings such as ours where the use of preterm milk fortifiers is an exception rather than the rule owing to the prohibitive cost and poor availability of such fortifiers. Also the high incidence of preterm deliveries in our settings makes the utility of this study more appealing.

SUMMARY AND CONCLUSIONS

- The lipid content of completely expressed hindmilk rich feeds is two to three times that of partially expressed milk feeds.
- This difference in fat composition can be exploited in feeding strategies to overcome the extreme variability of human milk lipid content and improve the weight gain of preterm newborns.
- In our settings newer and more effective strategies of preterm feeding that are simple, and easy to perform will be of great utility.
- Thus this technique of lacto-engineering own mother's milk to increase fat content, first proposed in the 1970's, can achieve this aim and needs more attention than it has been given in the past.

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PROFORMA USED FOR DATA COLLECTION

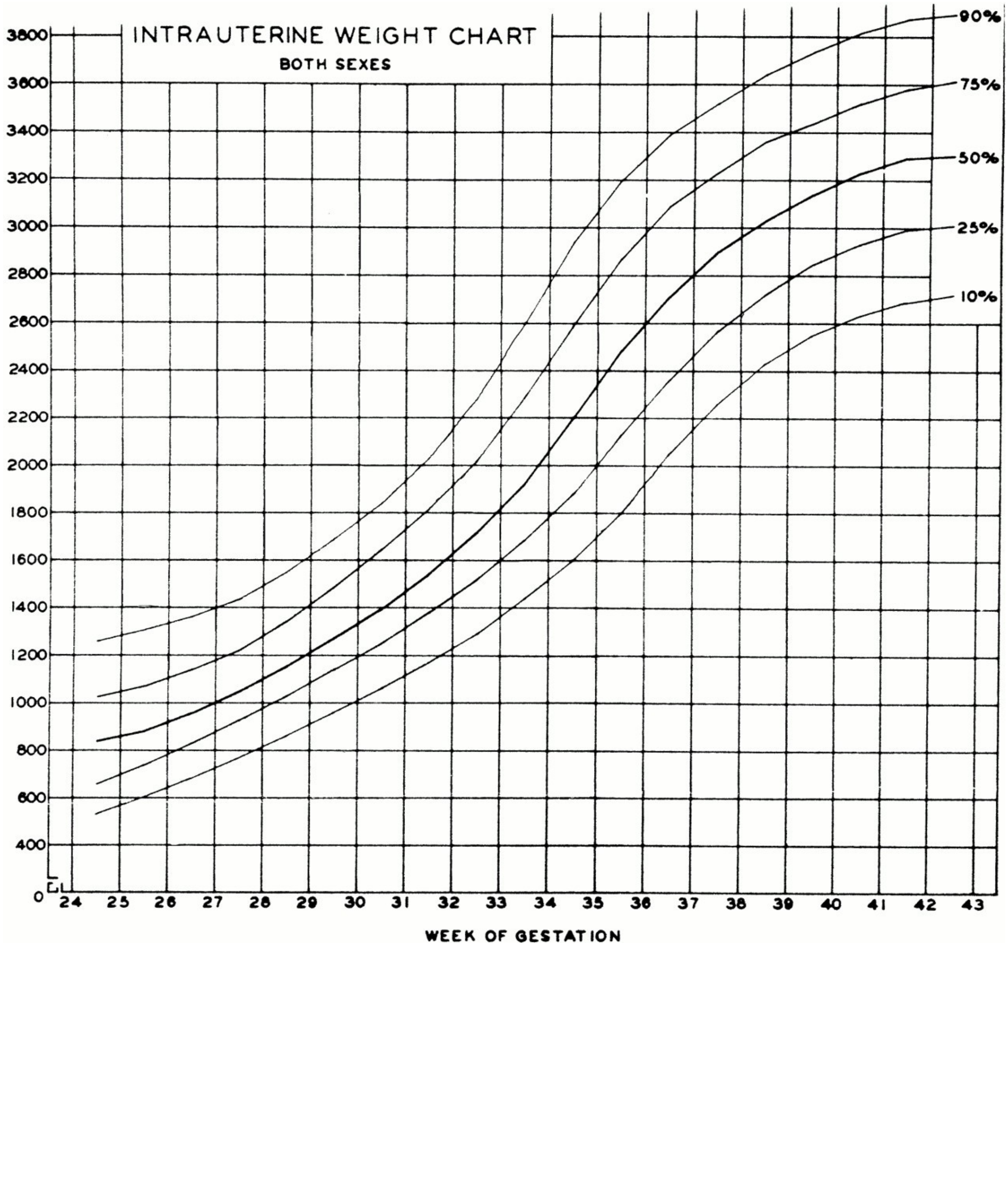
1. NAME OF THE NEWBORN
2. GESTATIONAL AGE ACCORDING TO NEW BALLARD'S SCORE
3. BIRTH WEIGHT
4. INITIAL WEIGHT ON DAY OF INCLUSION IN THE STUDY
5. CLASSIFICATION INTO LGA/AGA/SGA ACCORDING TO THE INTRAUTERINE GROWTH CURVES OF LUBCHENKO
6. NUMBER OF DAYS IN THE TRIAL
7. DAILY TOTAL FEED VOLUME
8. AVERAGE FEED VOLUME DURING THE PERIOD OF STUDY
9. DAILY WEIGHT ON EACH DAY OF THE STUDY
10. WEIGHT CHANGE IN gm/kg/day DURING THE STUDY PERIOD
11. DAILY FAT CONTENT OF MILK USED
12. AVERAGE FAT CONTENT OF MILK USED AS MEASURED BY CREAMATOCRIT

ABBREVIATIONS USED

LGA	–	LARGE FOR GESTATIONAL AGE
SGA	–	SMALL FOR GESTATIONAL AGE
AGA	–	APPROPRIATE FOR GESTATIONAL AGE
PDA	–	PATENT DUCTUS ARTERIOSUS
NEC	–	NECROTIZING ENTEROCOLITIS
IVH	–	INTRAVENTRIVULAR HEMORRHAGE
NICU	–	NEONATAL INTENSIVE CARE UNIT
LBW	–	LOW BIRTH WEIGHT
VLBW	–	VERY LOW BIRTH WEIGHT
ELBW	–	EXTREMELY LOW BIRTH WEIGHT
CRCT	–	CREAMATOCRIT

APPENDIX 2

LUBCHENCO INTRAUTERINE GROWTH CURVES



APPENDIX 3

BABSON AND BRENDA'S POST NATAL GROWTH CURVES